Cell selection by magnetic separation

<Spleen>

1. Get cells from mouse.

2. Collect cells into 15ml tube, centrifuge (1,500rpm, 4°C, 5mins).
3. Aspirate PBS, add 5ml of red cell lysis buffer to resuspend cells.
4. Centrifuge (1,500rpm, 4°C, 5mins). Then wash by 10ml of PBS.
5. Add 1ml of PBS to collect cells into Eppendorf tube.
6. Pick 10ul of cells, dilute x100 by PBS, counting.
7. Centrifuge the remained cells (1,500rpm, 4°C, 5mins).
8. Add 500ul of sorting buffer and 5ul of FcR blocking reagent (labeled as 2.4Gz), incubate at RT, 10mins.
9. Add 1ul of biotin, incubate at 4°C, 15mins.
10. Move cells to 15ml tube, add 10ml of PBS to wash (1,500rpm, 4°C, 5mins).
11. Aspirate supernatant, resuspend cells by 300ul of sorting buffer.
12. Move it to Eppendorf tube, add 2ul/1x10^7 cells of anti-biotin microbeads (vortex 30s before use), incubate it at 4°C, 15mins.
13. Wash cells twice by 500ul of PBS.
14. Add 500ul of sorting buffer to resuspend cells.
15. Correctly put MS columns on the magnet.
16. Rinsing columns by 500ul of sorting buffer.
17. Apply resuspended cells into columns flow-through in 50ml tubes.
18. Wash the cells by 500ul of sorting buffer, x3.
19. Remove columns from the magnet and place it on 15ml tubes.
20. Add 1ml of sorting buffer, then immediately flush out the magnetically labeled cells by firmly pushing the plunger into columns.